REVIEW ARTICLE

Manifold Molecular Signaling Targets of Tropolones in Multifarious Diseases

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Abstract

Tropolones are strewed phytochemicals having scaffold contains seven-membered ring system belongs to a family of Cupressaceae plants. Tropolone derivatives targeting multiple signaling transcription factors, cell cycle regulatory components, proteins, growth factors, kinases, and cytokines also involved in inflammatory mechanisms as well as involved in antioxidant, antimicrobial, anti-inflammatory, antithrombotic, and anticancer activities. To identify the therapeutically potent pharmacophores from the natural components is the long-term focus in pharmaceutical industry. Our preliminary findings on Hinokitiol (HIOL) showed binding potential in mitogen-activated protein kinase and ABL proto-oncogene 1 by *in silico* docking method perhaps in apoptosis of several cancers. This review aimed at gathering most of the available information to document the potential efficacy of tropolones and its molecular mechanisms. Although natural products usually have fewer activities compared with synthesized compounds, it will shed a light on studies aiming to discover new drugs from tropolone derivatives for targeting multiple signaling mechanisms for various diseases.

Key words: Tropolones and their derivatives, molecular mechanisms, Hinokitiol, docking studies

INTRODUCTION

The importance of natural products for human health and treatment of disease has been especially in scale throughout the industrialization of human evolution. Natural products possess potent pharmacological activity that regulates various signaling pathways to treat multifactorial diseases. Modern synthetic chemistry and combinatorial chemistry associated with the new technological tools such as genomics, proteomics, and metabolomics provide a wider platform to use of natural products.[1] Recent days, many natural compounds are developed and processed with potent pharmacological effects such as anti-infective,[2] anti oxidative,[3] anti-inflammatory,[4] and anticarcinogenic[5] properties. Hence, some of the natural compounds employed as lead compound to obtain high semi-synthetic pharmacological derivatives with increased efficacy for the therapeutic use. These are successfully declared as the knowledge to assemble from the past several decades will significantly inspire us to develop these natural products as novel potential drugs for future therapeutic use.[6]

Tropolone is a 2-hydroxy-2,4,6-cycloheptatrien-1-one. It is an isomer of benzoic acid. Chemically, it is a seven-membered, non-benzenoid aromatic molecule possessing three double bonds conjugated with a carbonyl group. Hence, tropolone is regarded as a vinylog of a carboxylic acid and considered as that the tropolone ring might be bioisosteric with benzoic acid. One of the tropolone derivative, Hinokitiol (HIOL), and its related derivatives and analogs with a tropolone skeleton have been displayed the versatile potent biological activity to treat various diseases such as antimicrobial,[7] antifungal,[8] cytotoxic effects on mammalian tumor cells,[9] anti-inflammatory,[10] antiviral,[11] antioxidant activity,[12] inhibition of platelet activation,[13] and neuroprotective effect.[14] These observations are the driving force for the further development of our research in this field. Moreover, the development of this group of

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compounds may lead better pharmacological profile than standard drugs and serve as templates for the construction of better drugs to various diseases. After studying the docking poses and binding modes of the docked compounds, the necessity of hydrogen bond formation for enhancing the activity of this class of compounds can be highly advocated.[15] The emergence and spread of antimicrobial resistance have become one of the most serious public health concerns across the world. The ever-growing resistance to antibiotics leads to continuous screening for new biologically effective compounds of either natural or synthetic origin. In recent years, environmentally benign reagents is one of the most fascinating development in the synthesis of organic compounds. Multicomponent reactions play an important role in combinatorial chemistry because of the ability to synthesize target compounds with greater efficiency and atom economy by generating structural complexity in a single step from three or more reactants.[16]

Tropolone and HIOL (β-thujaplicin) that are present in heartwood of several Cupressaceae trees are having several properties, other than that it is a well-known biochemical target. In the present studies, it was showed that tropolone and HIOL having important role in the pharmaceutical industry as active pharmaceuticals. The prevalence of tropolone derivatives in biologically active molecules has stimulated the need for efficient ways to make these heterocyclic leads. In view of these observations, it was thought worthwhile to update current knowledge in tropolone derivatives and its multiple target mechanisms for antimicrobial, anti-inflammatory, antithrombotic, anticancer activities, and its various signaling mechanisms.

METHODOLOGY OF THE REVIEW

The literature review was carried out from January 2020 to December 2020 using web search engines such as Google Scholar and Google, then publishing sites such as PubMed, Science Direct, Elsevier, and BioMed Central. The literature sources and databases were chosen based on the topic covered (i.e. tropolones and their derivatives, molecular mechanisms HIOL, beta-thujaplicin, uses, pharmacology, phytochemistry, cancer, and other therapeutic values). The following keywords were used to search the literature sources: HIOL, beta-thujaplicin, carcinogenesis, antioxidant, and molecular docking studies.

Role of tropolones in transcription factors and inflammatory cytokines

Nuclear factor kappa B (NF-κB) is a transcription factor which has an essential role in inflammation. It has been demonstrated that NF-κB promotes immunity by controlling the expression of cytokines and chemokines involved in inflammation.[17] Cytokines such as tumor necrosis factor (TNF)-alpha interleukin (IL) 1 to initiate a signaling cascade resulting in the direct activation of NF-κB resulting in amplifying inflammation involved in several autoimmune disorders. NF-κB drives expression of target genes that mediate cell proliferation and release of antimicrobial molecules and cytokines to activate the immune response, thereby augmenting the duration of chronic inflammation.[18]

Chronic infections, inflammatory bowel disease, parkinsonism, and pancreatitis are linked to inflammation. Several molecular mechanisms are involved in the inflammation due to oxidative stress which activates the transcription factors, for example, NF-κB.[19] Signal transducers and activators of transcription 3 (STAT3). Several cytokines, for example, IL2, IL6, and IL22 are also involved in the inflammation. It is well documented that inhibition of cytokines and transcription factors may play a major role for potent anti-inflammatory property. Recent study demonstrated that HIOL showed the protective potential against MIA-induced chondrocytes through the inhibition of matrix metalloproteinases (MMP-1), 3, 13 and β-catenin, thereby protecting cartilage degeneration.[20] β-thujaplicin inhibited lipopolysaccharide-induced PGE2, IL-6, and TNF-α production as well as iNOS, COX2, and NFκB protein in RAW 264.7 macrophage cell lines.[10]

HIOL also reduced interferon gamma secretion, upregulated p21 expression in ConA-activated T lymphocytes, and also downregulated cyclin D3, E2F1, and cyclin-dependent kinase 4 (CDK4) expression which revealed that HIOL may regulate immune responses by arresting cell cycle at the G0/G1 phase involved in autoimmune disorders.[21] Antioxidant is very important treatment for various diseases caused by the damage of oxidative stress. The antioxidant effect of HIOL on the ultraviolet B-induced apoptosis in mouse keratinocytes ascribed to the induction of metallothionein gene expressions.[22]

Tropolone derivatives and HIOL protect cells by modulating the effects of oxidative stress which plays an important role in inflammatory-mediated disorders and also in carcinogenesis. It has been well documented from our previous studies that by hampering the oxidative stress, perturbations may regulate the inflammatory mechanisms.[23] HIOL has potential to inhibit the inflammatory reaction through its antioxidant potential in HCE cells against H_2O_2 by modulating antioxidant enzymes SOD, CAT, total antioxidant capacity, and antiapoptotic pathway by Bcl-2 downregulation and Bax upregulation.[24] Further, HIOL showed a significant protective effect against ocular surface inflammation through inhibiting the NF-κB pathway involved in surface inflammation of dry eye syndrome.[25]

HIOL acts against the bacteria which are confirmed through the inhibition of expression levels of COX-2 and IL-1. HIOL-modified calcium silicate cement (root end filling material)

through inhibited the expression level of inflammatory cytokines but also had better cytocompatibility. Therefore, the HIOL-modified calcium silicate cement may be a potential root end filling material for clinic which indicates that the compound having potent antibacterial and anti-inflammatory effects.[26]

Role of tropolones in proliferative and antithrombotic mechanisms in vascular smooth muscles

Atherosclerosis is accompanied chronic vascular abnormality which causes the majority of cardiovascular diseases. The growth of vascular disease involved with combination of excessive lipid deposition in the intima, endothelial dysfunction, proliferation of vascular smooth muscle cells (VSMCs), exacerbated innate and adaptive immune responses, and remodeling of the extracellular matrix, resulting in the formation of an atherosclerotic plaque.[27] Atherosclerosis has the potential to promote the development of thrombotic disorders in the venous system. At the same time vitalized the activity of coagulation process and inflammatory process in both venous and arterial systems. The interaction between the coagulation factors and atherosclerotic plaque components, platelet receptors are leading to platelet activation and the subsequent formation of atherothrombosis. Platelet activation plays a major role in atherosclerosis.[28] HIOL blocks the specific signaling pathway involved in platelet activation through inhibiting the PLCγ2 and PKC cascades as well as hydroxyl radical formation followed by inhibiting the activation of Akt and mitogen-activated protein kinase (MAPK) and simultaneously inhibits platelet aggregation. These indicate that HIOL may have a potent therapeutic value to prevent the thromoembolism and it may the treatment of thromboembolic disorders.[29]

VSMCs perturbations revealed in simulation of inflammatory cytokine signaling cascades by which the abnormal proliferation and migration of VSMCs occur and thereby lead to atherosclerosis. Several reports documented that PDGGF-BB which is a potent mitogen plays a key role in atherosclerosis. Recent studies have shown that it can induce VSMCs proliferation through the activation of c-Jun N-terminal kinases (JNK) and PLC-γ1. Further, it has been documented from our previous studies that JNK pathway was involved in apoptosis through the oxidative stress mechanism.[30] HIOL inhibits PDGF-induced VSMC proliferation through suppressing JNK1/2 and PLC-γ1 phosphorylation and limiting the synthesis of specific cell cycle enzyme, PCNA, as well as stimulating p27kip1 expression which, in turn, prevents the abnormal proliferation of VSMCs by inducing cell cycle arrest at the G0/G1 phase. It may offer a new insight into the anticardiovascular agents of HIOL.[31]

Role of tropolones in antimicrobial mechanisms

The demand for new antiviral agents has increased evidently in recent years. There are many contributing factors to this increased demand due to the antigenic variations of several pathogenic chronic viral infections such as HIV and hepatitis B and C like and emergence of new viruses such as the SARS coronavirus. The discovery of the inhibitors of the HSV and the HIV-1 infections is clearly bright examples that allow treating millions of infected patients. The antiviral drug discovery has already obtained several magnificent triumphs in HIV-1, HIV-RT 2, and HBV RNase H targets using tropolones are candidates for the development of novel antiviral drugs.[32]

Beta-thujaplicinol is act as an antiviral compound against HRV, coxsackievirus, and mengovirus infections, pyrrolidinedithiocarbamate reduces coxsackievirus B3 replication through inhibition of the ubiquitin proteasome pathway. It also inhibits the RNase H activity of HIV-1 and HIV-2 RT at submicromolar concentrations without affecting DNA polymerase function of enzyme through interaction between the cytosine in the overhang of the viral DNA and the Q148 occurs after a conformational change of the integrase-viral (donor) DNA complex.[33]

Thujaplicin-copper chelates inhibit influenza virus-induced apoptosis of Madin-Darby Canine Kidney ((MDCK) cells. Furthermore, the copper chelates inhibited the release of the viruses from the infected MDCK cells during apoptosis. It suggests that the copper chelates affect MDCK cells directly in the early stage of influenza virus-induced apoptosis.[34] Tropolone derivatives used as an antimicrobial agent plausible candidate to inhibit the synthesis of the capsular polysaccharide of pathogenic strains.[35] Such as thujaplicin, which has action in a preparation combined with ZnO, can reduce the number of viable bacterial cells on the skin surface of atopic dermatitis it indicates that HIOL having potential effect on treating eczematous lesions of atopic dermatitis.[36] It has been well documented that HIOL reduced both antibacterial *E. coli* and antifungal *S. aureus* cellular respiration, nucleic acid synthesis, and protein synthesis.[37] Ras, cAMP, and PKA signaling play an important role in pathogenesis of *Candida albicans* and orchestrates the expression of distinct but interrelated traits. Ras1 and Cyr1 *Candida albicans* proteins that play a role in cell adhesion, biofilm formation, and filamentation required for pathogenesis. HIOL inhibited *C. albicans* adenylate cyclase 1 (CYR1) and RAS1, which function in the regulation of cell growth due to the interruption of RAS-signal transmission, such as the cAMP pathway indicates antimicrobial potential.[38]

MITF is an important transcriptional regulator of tyrosinase, which plays a major role in melanin synthesis.[39] Current report indicates that the extracellular signal-regulated kinase (ERK) signaling pathway is an important for regulation of melanogenesis. Hence, ERK activation stimulates MITF phosphorylation as well as subsequent degradation.[40,41] In HIOL, we found that ERK activation induces MITF phosphorylation and degradation, which lead to a reduced

tyrosinase level and decreased melanogenesis and also suppresses both human and mushroom tyrosinase activity and reduced MITF, and subsequently tyrosinase protein production, it reduces the melanin synthesis in Mel-Ab cells. Moreover, the combination of epigallocatechin gallate and HIOL acted synergistically indicates the strong tyrosinase inhibitory activity.[42]

Role of tropolones in signal transduction cascades and cell cycle arrest mechanisms in tumor cells

Epidermal growth factor receptor (EGFR) pathway is related with process of cirrhosis to mortality. The EGFR is the first identified target to develop the novel anticancer agents. Particularly, in breast cancer, EGFR levels are overexpressed. The current studies show that EGFR and its downstream pathway regulate epithelial-mesenchymal transition, tumor invasion, and migration. This study indicates that EGFR-targeted therapy may play a crucial role to prevent metastases.[43] HIOL downregulates EGFR protein expression in mammosphere-forming breast cancer stem cell (BCSCs) without affecting the expression of messenger RNA, and the protein stability of EGFR in BCSCs was also decreased by inhibiting the vasculogenic mimicry activity of BCSCs through stimulating proteasome-mediated EGFR degradation which indicates that HIOL has antivasculogenic mimicry agent, and may be useful for the development of novel breast cancer therapeutic agents.[44]

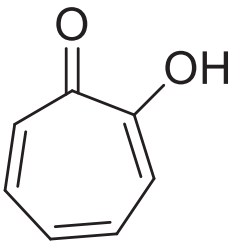
The p53 protein is the mostly mutated tumor suppressor gene in cancer, it is responsible for a critical cellular function, which may results in cell cycle arrest, repairing the damaged DNA, cellular apoptosis, senescence, metabolic changes, or autophagy.[45] P53 translocates to the mitochondrial surface which is directly binds to Bcl-2 its leading to apoptosis.[46] HIOL possesses potent anticancer effects against lung adenocarcinoma cells. It inhibits the proliferation by inducing the p53-independent DNA damage response, autophagy, S-phase cell cycle arrest, senescence, and colony formation ability of lung adenocarcinoma cells as well as the EGFR-TKI-resistant lines PC9-IR and H1975. HIOL inhibited the growth of xenograft tumors in association with DNA damage and autophagy it supports the potential of this naturally occurring compound as a therapeutic agent in lung cancer treatments.[47]

Autophagy induced due to the pathogen infection, nutrient starvation, aggregation of toxic proteins, and mitochondrial depolarization. Hence, phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR pathway is ingrained suppresses the PI3K/Akt signaling pathway because of hyponutrient microenvironment which leads to autophagy induction in tumor cells.[48] HIOL can induce cell death through an autophagic pathway by inhibition of tumor cells. HIOL increased autophagy marker in tumor cells and immunoblotting revealed that the levels of phosphoprotein kinase B (P-AKT), phosphomammalian target of rapamycin (P-mTOR), and also decreased the levels of phospho-p70 ribosomal s6 kinase (P-p70S6K) in tumor cells which indicate that HIOL induces the autophagic signaling pathway through downregulation of the AKT/mTOR pathway in B16F10 cells. It shows that HIOL may control tumor growth by inducing autophagic signaling pathway.[49]

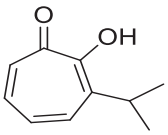
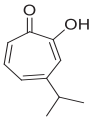
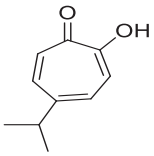
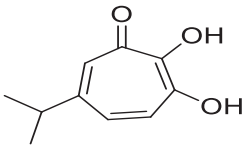
MMPs are involved in the tumor progression to processing invasion of tumor cells into surrounding tissue and metastasis which are the significant checkpoints for carcinogenesis. Moreover, MMPs exhibiting the most significant upregulation in tumor cells. Moreover, MAPKs play a major

Table 1: Biologically active tropolones

Scaffold



Tropolone (2-Hydroxytropone)

Side chain	Compound
C_3 carbon-Propyl	
	α-thujaplicin(3-Isopropyltropolone)
C_4 carbon-Propyl	
	β-thujaplicin(Hinokitiol) (4-Isopropyltropolone)
C_5 carbon-Propyl	
	γ-thujaplicin (5-Isopropyltropolone)
C_6 carbon-Propyl C_3 carbon-Hydroxyl	
	hujaplicinol(2,7-Dihydroxy-4-isopropylcyclohepta-2,4,6-trien-1-one)

role in the biological growth processing of tumor cells.[20] The expression of MAPK correlates with the proliferation of cancer cells, which leads to the metastasis of tumor cells but it is not affected by outside stimuli. However, when stimulated by mitogens such as growth factors, MAPK expression increases significantly and has regulatory effects on multiple important pathophysiological operation including cellular growth, differentiation, stress, adaption to the environment, and the inflammatory response of tumor cells.[50]

The NF-kB is the one of the transcription factors involved in the regulation of various biological responses and the key function for the regulation of immune responses and inflammation as well as plays a major role in oncogenesis. NF-kB regulates gene expression involved in development and progression of cancer such as migration, apoptosis, and proliferation. Aberrant NF-kB activation displayed in human malignancies which is a driving force to elucidate the signaling mechanism and also functional consequences of NF-kB activation. Hence, NF-kB signaling pathway becomes an important role for the clinical therapy and it may use to develop new way to targeted anticancer therapy.[51] HIOL inhibits metastasis by reducing the expression of MMP-1 activation and by suppressing the phosphorylation of MAPK signaling molecules such as ERK 1/2, p38 MAPK, and JNK.[31] HIOL treatment reversed IκB-α degradation and inhibited the phosphorylation of p65 NF-κB and cJun in B16-F10 cells. And also, HIOL suppressed the translocation of p65 NF-κB from the cytosol to the nucleus which indicates reduced NF-κB activation.[52]

MMPs are a group of proteolytic enzymes which are crucial in degradation of basement membrane and extracellular matrix that are fundamental for cancer cell invasion and metastasis. MMP9 stimulates increased cell proliferation and also initiates apoptosis by causing loss of contact of cells to the basement membrane.[53] HIOL exerts anticancer effect through downregulation of proteolytic enzymes such as MMP-9 and -2 as well as enhanced the antioxidative enzymes SOD and CAT and also inhibiting OH· radical formation. HIOL also restored melanoma-induced degradation of elastic fibers and collagen in the lungs.[54] These results may accelerate that the HIOL may act novel therapeutic agent for the treatment of malignant cancers.

CDK having a positive impact on the facing challenges against hormone therapy of estrogen receptor (ER). CDK4/6 is the target for developing advanced effective drugs which is recently entered into the clinical trials for the ER-positive breast cancers. CDK cooperates with many transcription factors, which enhancing the activity through upregulation in breast cancer as well as associated with tumor progression and inversely associated with expression of ER which affects ER signaling. Hence, it suggests that CDK inhibitor may explore for the first-line therapy in future clinical development of CDK targeting drugs.[44] β-thujaplicin suppressed the proliferation through arresting the cell cycle transition from G1 to S phase as well as inhibited the expression of cell cycle-related proteins, cyclin D1, and CDK4 in MCF-7 and T47D luminal subtype and also downregulated the ER-α through enhanced proteolysis by ubiquitination, which led to cell growth inhibition breast cancer cells. It indicates that β-thujaplicin may have a potent agent regulating the hormone sensitive mammary tumorigenesis.[55]

The Bcl2 is the key role to regulate the intracellular apoptosis which is important for cancer development. It promotes cell migration, cell death, and invasion in cancer cells. The important noticed statement is that Bcl2 is one of the potential targets which contains miR-125b-5p binding site at its 3′UTR region. miR-125b-5p downregulates Bcl2 in tumor cells. Apoptosis of the tumor cells was similar in cells with or without miR-125b-5p overexpression. p21 inhibits cell cycle progression primarily through the inhibition of CDK2 activity.[56] p21 expression positively correlates with the suppression of genes that are important for cell cycle progression and the induction of genes associated with senescence. P21 suppresses E2F1-dependent Wnt4 expression, thereby controlling cellular growth. P21 also binds to represses the transcription factor STAT3, thereby inhibiting cytokine-stimulated and STAT3-dependent gene expression. P21 can protect against apoptosis in response to other stimuli such as those induced by growth factor deprivation. HIOL-induced S-phase arrests in the cell cycle progression and decreased the expression levels of cyclin A, cyclin E, Bcl-2, and Cdk2 simultaneously increased the expression of P21, a Cdk inhibitor, Bax, cleaved caspase 9, and 3[57] which suggests that

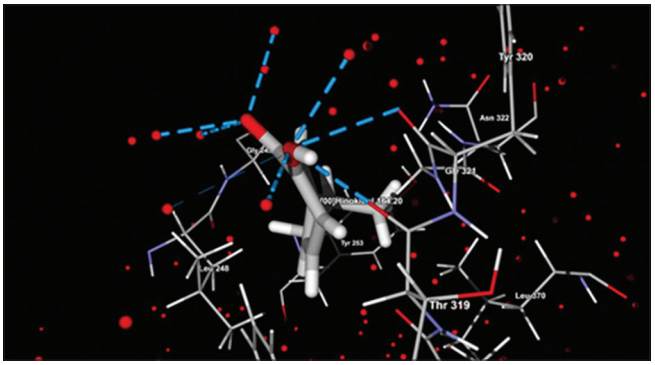


Figure 1: Docked view of Hinokitiol against 2HZI captured using ligand energy inspector tool in Molegro Virtual Docker

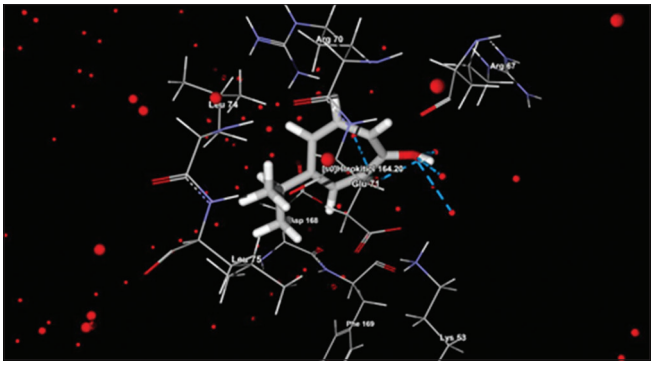


Figure 2: Docked view of Hinokitiol against 3LFF captured using ligand energy inspector tool in Molegro Virtual Docker

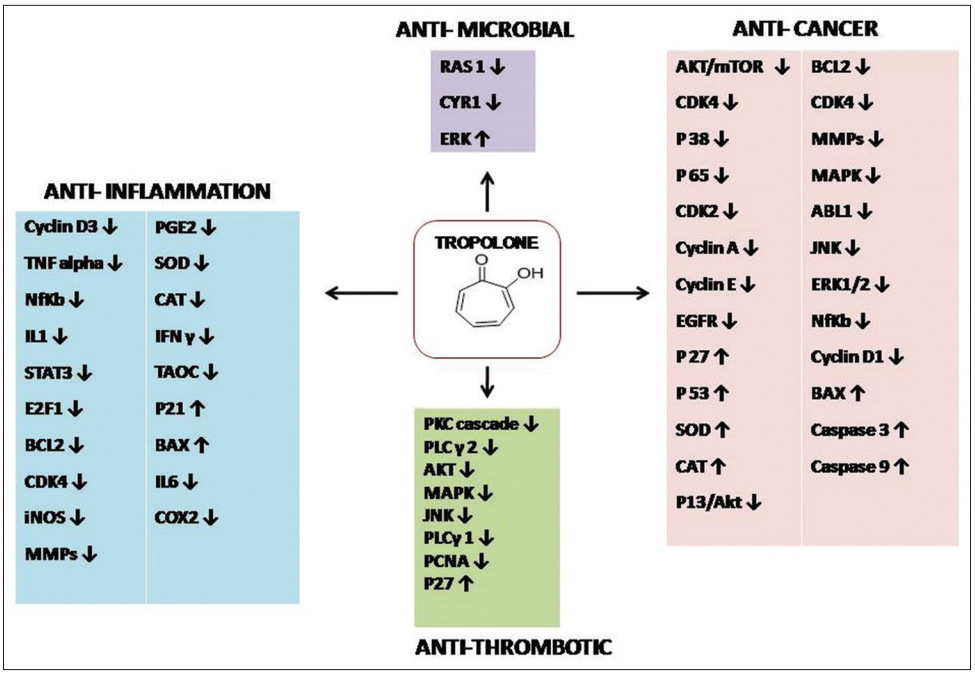


Figure 3: Molecular signaling targets of tropolones

the suppression of tumor formation by HIOL in human colon cancer may occur through cell cycle arrest and apoptosis.[58]

HIOL-induced growth inhibition of G1 cell cycle arrest with blocking the G1-S-phase transition and increased P27 by inhibiting P27 phosphorylation at Thr (187) and by downregulating Skp2 expression andP21 and also decreases the levels of Cdk2, cyclin E, and phosphorylated Rb. It also increasing Cdk inhibitor levels and decreasing cyclin A expression in FEM human melanoma cells through inhibition of cell growth and DNA synthesis. These therapeutic potential of HIOL may lead to novel cell cycle-based anticancer strategies for malignant melanoma.[56]

HIOL suppresses androgen/AR-mediated cell growth and androgen-stimulated DNA synthesis by [(3)H]thymidine. HIOL suppresses the intracellular and secreted PSA levels on prostate cancer. The ligand-binding assay of HIOL blocks binding of the synthetic androgen [(3)H]R1881 to AR in LNCaP cells. These results proved that the HIOL having potent effective against prostate cancer and it may become a novel chemopreventive or chemotherapeutic agent for prostate cancer.[59] The safety profile of HIOL zinc ionophore having the potent therapeutic effect for COVID-19. HIOL suppressing the viral replication by impairing viral polyprotein processing this is due to zinc ions.[60]

The search for new anticancer drugs through protein ligand interactions and the development of more effective treatment strategies continues to be imperative in the present scenario due to the molecular multidrug resistance of both matured cancer cells and cancer stem cells. Successful application toward reckoning approaches through molecular docking score has the power to screen hits from a huge database and design novel small molecules. Molecular docking studies display an crucial role in the judicious drug design and development. Recent work revealed that tropolone showed potent inhibitory effect against human tyrosinase through homology modeling and molecular docking which encourages us to select the potential targets of human proto-oncogene tyrosine-protein kinase (ABL proto-oncogene 1) and MAPK to dock with the tropolone derivative HIOL. Based on the MolDock score, rerank score, and lipin rule, the HIOL having good interaction with receptor which is shown in Figures 1 and 2.

CONCLUSION

Multiple targeting potentials make it a promising agent for the prevention and treatment of various diseases. Many of these signaling pathways appear to be vulnerable to targeting with natural compounds. Numerous *in vitro* and *in vivo* studies have demonstrated that these phytochemicals can modulate the signal pathways of various diseases. This review also highlights the fact that the tropolone derivatives [Table 1] have a diverse range of molecular targets, supporting the concept that it interacts with numerous molecular targets [Figure 3]. Consequently, the synthesis of analogues of the tropolone through reckoning could help raise the number of targets affected by biological molecule and better delineate the pathways of action, opening new perspectives in the search, and synthesis of novel agents to treat several diseases. Tropolones on human clinical trials are relatively limited. However, the existing published research suggests that it may offer considerable potential as an aid to treating or preventing

multifactorial disorders. Nevertheless, more randomized clinical trials are needed to solidify our understanding of its therapeutic potential.

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